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5 February 1988

Dr. William O. Berry AFOSR/NL Bolling Air Force Base, D.C. 20332-6448

Dear Dr. Berry:

I am happy to submit this Final Technical Report for the Equipment Grant (AFOSR-87-0027) made by the AFOSR to my laboratory for the period 00/1/86 - 9/30/87. The size of the grant was \$72,500; this was supplemented, as per agreement, by additional funds (\$7,000) granted to M.I.T. by a foundation.

The funds were used to purchase a multi-detector liquid chromatography system consisting of:

BioRad AS-48 refrigerated automatic injector \$ 7,500

Hewlett-Packard HP1090 with workstation,
software, and fast UV-Vis spectral detector 48,500

Berthold LB 506-C radioisotope detector 16,500

ESA 5100A Coulochem detector 5,500

This latter item represented the only significant departure from our original proposal; it was described in a letter that I sent to your office after that proposal was approved. We reduced the funds to be spent on the HP workstation system in order to purchase this supplementary detector, which should increase the selectivity of our assays.

The major equipment was installed in the summer of 1987. It has been used to compare several methods of amino acid analysis. At present, we are using pre-column derivitization with OPT with full-spectrum detection. Integration of signals for reports can be carried out at any wavelength; 266 and 338 nm give us the most useful chromatograms. An additional derivitizing agent, FMOC, allows the detection of secondary amines (e.g., proline), but leads to a significant number of by-product peaks. Since our main interest is in the primary amino acids, we usually eliminate this step. PITC derivitization will be considered in the future.

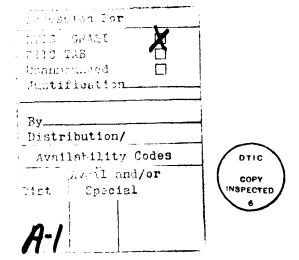
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The initial studies have focussed on resolving threonine, glycine, and histidine peaks for a study of CSF samples. Work is beginning on our project on amino acid analysis of retinal superfusates (and, possibly, tissues). Since our proposal was submitted, the initial results (which led to a doctoral thesis) showed an interesting interaction between tyrosine and light. The experimental plan has therefore been extended to include superfused cells in culture to determine whether they also respond to light or whether an intact retina is required. We will test these cell superfusates in hopes that our system is sensitive enough to detect amino acids in them as well.

Two of the requested detectors arrived much earlier than the bulk of the equipment and were thus available for use in additional projects involving synthesis and release of neurotransmitters and related compounds. The coulochem detector has been heavily used for measuring dopamine, DOPAC, and HVA in samples from the <u>in vivo</u> dialysis studies on tyrosine and phenylalanine administration. As you know, this work has resulted in numerous publications and presentations at scientific meetings. The radioisotope detector has been utilized to monitor choline and its metabolites formed from labelled choline or methionine in cell cultures or brain slices; this system has also led to publications and presentations.

Thank you and the AFOSR again for making this research possible.

Cordially yours,



Richard J. Wurtman, M.D.